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REPLY TO FIRST WRITTEN OPINION

VIA FACSIMILE

Mail Stop PCT, Attn: IPEA/US
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

This is a reply to the First Written Opinion mailed from the International Preliminary Examining Authority on 07 July 2004 for the subject application.

Claim Amendments

Claim 17 has been amended to depend from Claim 7.

In accordance with PCT Rule 66.8(a), replacement page 48 is attached.

Concerning Item V: Reasoned Statement With Regard to Novelty, Inventive Step and Industrial Applicability

Novelty

Claims 1-11 have been rejected under PCT Article 33(2) as lacking novelty in view of Sheriff *et al.* (*Peptides*, 75-76:309-318 (1998)). In particular, the Examiner

contends that Claims 1-11 are anticipated by Figures 2 and 3 of the Sheriff *et al.* reference. Applicant respectfully disagrees that Claims 1-11 are anticipated by the Sheriff *et al.* reference.

In Figures 2 and 3, Sheriff *et al.* teach a method in which human neuroblastoma cells were transfected with a vector containing a CRF-luciferase fusion gene, followed by treatment with neuropeptide Y (NPY) (100 nM). Luciferase activity after treatment with NPY was monitored and compared to luciferase activity in untreated control cells (basal).

Sheriff *et al.* also teach methods in which human neuroblastoma cells were transfected with a vector containing a CRF-luciferase fusion gene, followed by treatment with NPY (100 nM), forskolin (10 μM) and/or thapsigargin (10 μM). Luciferase activity after treatment was monitored and compared to untreated control cells (basal).

In contrast, Claims 1-11 relate to methods for identifying candidate compounds for enhancing CREB pathway function comprising:

(a) contacting cells comprising an indicator gene operably linked to a CRE promoter with a test compound and with a suboptimal dose of a CREB function stimulating agent;

(b) determining indicator activity in the cells which have been contacted with the test compound and with the CREB function stimulating agent;

(c) comparing the indicator activity determined in step (b) with the indicator activity in control cells which have been contacted with the CREB function stimulating agent and which have not been contacted with the test compound;

(d) selecting said test compound if: (i) the indicator activity determined in step (b) is increased relative to the indicator activity in the control cells which have been contacted with the CREB function stimulating agent and which have not been contacted with the test compound; and (ii) the indicator activity in control cells which have not been contacted with the CREB function stimulating agent and which have been contacted with the test compound is not significantly different relative to the indicator activity in control cells which have not been contacted with the CREB function stimulating agent and which have not been contacted with the test compound;

(e) repeating steps (a) to (d) with a range of different concentrations of the test compound selected in step (d); and

(f) selecting the test compound if: (i) the indicator activity is increased in the range of concentrations for the test compound relative to the indicator activity in the control cells which have been contacted with the CREB function stimulating agent and which have not been contacted with the test compound; and (ii) the indicator activity in control cells which have not been contacted with the CREB function stimulating agent and which have been introduced the range of different concentrations of the test compound is not significantly different relative to the indicator activity in control cells which have not been contacted with the CREB function stimulating agent and which have not been contacted with the test compound.

The methods disclosed by Sheriff *et al.* in Figures 2 and 3 do not include any of steps (a) to (f) recited in independent Claim 1. Of particular note, although in particular embodiments Sheriff *et al.* disclose treatment of cells with a CREB function stimulating agent (i.e., forskolin), the authors do not teach treatment with a *suboptimal dose* of the CREB function stimulating agent. Thus, Sheriff *et al.* do not teach or suggest a method comprising steps (a) to (f) recited in Claim 1. As such, Claims 1-11 must be considered novel over Sheriff *et al.*

Claims 12-16 have been rejected under PCT Article 33(2) as lacking novelty over Ying *et al.* (*J. Biol. Chem.*, 272:2412-2420 (1997)). In particular, the Examiner contends that Claims 12-16 are anticipated by Figure 9 of the Ying *et al.* reference. Applicant disagrees that Claims 12-16 are anticipated by the Ying *et al.* reference.

In Figure 9, Ying *et al.* teach transfection of Calu-6 and JEG-3 cells with a HREN CRE-luciferase fusion construct, a nonfunctional CRE mutant luciferase fusion construct or a cAMP-responsive somatostatin promoter (SSCRE) luciferase fusion construct alone. In Figure 9, Ying *et al.* also teach co-transfection of Calu-6 and JEG-3 cells with one of the luciferase fusion constructs along with a cPKA expression vector and either a wild-type or dominant negative mutant CREB-1 expression vector. Relative luciferase activity

was measured to assess whether transcriptional PKA response of the HREN promoter was mediated via a CREB-1-dependent mechanism.

Ying *et al.* also teach transfection of Calu-6 and JEG-3 cells with a HREN CRE-luciferase fusion construct, a nonfunctional CRE mutant luciferase fusion construct or a cAMP-responsive somatostatin promoter (SSCRE) luciferase fusion construct, followed by treatment with forskolin, as well as co-transfection of Calu-6 and JEG-3 cells with one of the luciferase fusion constructs along with a CREB-1 expression vector, followed by treatment with forskolin. Relative luciferase activity was measured to assess the ability of CREB to mediate cAMP response from the HREN promoter.

Calu-6 cells were derived from a human pulmonary carcinoma and JEG-3 cells were derived from a human placenta choriocarcinoma.

In contrast, Claims 12-16 teach methods for assessing the effect on CREB-dependent gene expression of a candidate compound for enhancing CREB pathway function comprising:

(a) contacting cells of neural origin with a candidate compound and with a suboptimal dose of a CREB function stimulating agent;

(b) assessing endogenous CREB-dependent gene expression in the cells which have been contacted with the candidate compound and with said CREB function stimulating agent; and

(c) comparing endogenous CREB-dependent gene expression assessed in step (b) with endogenous CREB-dependent gene expression in control cells which have been contacted with said CREB function stimulating agent and which have not been contacted with the candidate compound.

Ying *et al.* do not teach a method comprising steps (a) to (c) recited in independent Claim 12. Of particular note, Ying *et al.* do not teach or suggest a method in which cells of neural origin are contacted with a candidate compound and a CREB function stimulating agent. Additionally, although in particular embodiments Ying *et al.* disclose treatment of the non-neural Calu-6 and JEG-3 cells with a CREB function stimulating agent (i.e., forskolin), the authors do not teach treatment with a *suboptimal*

dose of the CREB function stimulating agent. As such, Claims 12-16 must be considered novel over Ying *et al.*

Claims 17-18 have been rejected under PCT Article 33(2) as lacking novelty over Kang *et al.* (*Cell*, 106:771-783 (2001)). In particular, the Examiner contends that Claims 17-18 are anticipated by Figures 3-5 of the Kang *et al.* reference.

Claim 17 has been amended to depend from Claim 7. Thus, Claims 17-18 as amended, include steps (a) to (f) of independent Claim 1 and steps (g) to (i) of dependent Claim 7, along with steps (a) to (d) of dependent Claim 17. Applicant submits that as amended, Claims 17-18 are not anticipated by the Kang *et al.* reference.

In Figure 3, Kang *et al.* teach a method in which the role of CaMKIV in LTP induction is examined by administering theta burst stimulation to dnCaMKIV transgenic mice.

In Figure 4, Kang *et al.* teach behavioral training of dnCaMKIV transgenic mice using a hidden platform version of the Morris water maze to assess the role of CaMKIV in hippocampus-dependent learning and memory.

In Figure 5, Kang *et al.* teach behavioral training of dnCaMKIV transgenic mice using a fixed location/visible platform version of the Morris water maze to assess the role of CaMKIV in hippocampus-dependent learning and memory.

In contrast, as amended, Claim 17-18 teach methods for assessing the effect on long term memory formation in an animal of a candidate compound for enhancing CREB pathway function comprising:

(a) administering to the animal the candidate compound to be assessed, said candidate compound identified according to the method of Claim 7;

(b) training the animal administered the compound under conditions appropriate to produce long term memory formation in the animal;

(c) assessing long term memory formation in the animal trained in step (b); and

(d) comparing long term memory formation assessed in step (c) with long term memory formation produced in the control animal to which the candidate compound has not been administered.

Kang *et al.* do not teach a method in which a candidate compound for enhancing CREB pathway function is identified using a method comprising steps (a) to (f) of Claim 1 and steps (g) to (i) of Claim 7. Thus, Kang *et al.* do not teach a method which comprises steps (a) to (f) of Claim 1, steps (g) to (i) of Claim 7, and steps (a) to (d) of Claim 17. As such, Claims 17-18 must be considered novel over Kang *et al.*

Claims 19-25 have been rejected under PCT Article 33(2) as lacking novelty over Son *et al.* (*J. Neurochemistry*, 79:1013-1021 (2001)). In particular, the Examiner contends that Claims 19-25 are anticipated by Figures 1-4 of the Son *et al.* reference. Applicant disagrees that Claims 19-25 are anticipated by the Son *et al.* reference.

In Figure 1, Son *et al.* teach a method in which hippocampal progenitor HiB5 cells were transfected with a GRE-luciferase reporter plasmid followed by treatment with vehicle (0.1% ethanol) or dexamethasone (DEX) (1 μM) and/or RU486 (5 μM). DEX is a synthetic glucocorticoid; RU486 is a glucocorticoid receptor (GR) antagonist. Luciferase activity after treatment with DEX was compared with luciferase activity after treatment with vehicle or RU486 and after simultaneous treatment with RU486.

In Figure 2, Son *et al.* teach a method in which HiB5 cells were treated with various concentrations of DEX or vehicle followed by treatment with PDGF (30 ng/mL). Cells treated with 1 μM DEX or vehicle were simultaneously treated with RU486 (5 μM). The morphological effects (i.e., neurite outgrowth) in the DEX and vehicle treated cells were quantified and compared.

In Figure 3, Son *et al.* teach a method in which HiB5 cells were treated with DEX or vehicle followed by treatment with PDGF. The mRNA levels of nestin and midsize neurofilament (NF-M) in the DEX and vehicle treated cells were examined and compared.

In Figure 4, Son *et al.* teach a method in which HiB5 cells were transfected with a CRE-luciferase reporter plasmid or AP-1-luciferase reporter plasmid, followed by treatment with PDGF in the presence of vehicle or 1 μM DEX. Luciferase activity after treatment with PDGF in the presence of DEX was compared with luciferase activity after treatment with PDGF in the presence of vehicle (i.e., in the absence of DEX) and after treatment with neither PDGF nor DEX.

In contrast, Claims 19-25 relate to methods for screening a compound for its ability to enhance CREB pathway function comprising:

(a) contacting cells comprising an indicator gene operably linked to a CRE promoter with a test compound to produce a test sample;

(b) contacting the test sample with a suboptimal dose of a CREB function stimulating agent;

(c) determining indicator activity in the cells which have been contacted with the test compound and with the CREB function stimulating agent;

(d) comparing the indicator activity determined in step (c) with the indicator activity in control cells which have been contacted with the CREB function stimulating agent and which have not been contacted with the test compound;

(e) selecting the test compound if: (1) the indicator activity determined in step (c) is increased relative to the indicator activity in the control cells which have been contacted with the CREB function stimulating agent and which have not been contacted with the test compound; and (2) the indicator activity in control cells which have not been contacted with said CREB function stimulating agent and which have been contacted with the test compound is not significantly different relative to the indicator activity in control cells which have not been contacted with said CREB function stimulating agent and which have not been contacted with said test compound;

(f) repeating steps (a) to (e) with a range of different concentrations of the test compound selected in step (e); and

(g) selecting said test compound if: (1) the indicator activity is increased in the range of different concentrations for the test compound relative to the indicator activity in the control cells which have been contacted with said CREB function stimulating agent and which have not been contacted with the test compound; and (2) the indicator activity in control cells to which have not been contacted with the CREB function stimulating agent and which have been introduced the range of different concentrations of the test compound is not significantly different relative to the indicator activity in control cells which have not been contacted with said CREB pathway function stimulating agent and

which have not been contacted with the test compound, thereby selecting a candidate compound;

(h) contacting cells of neural origin with the candidate compound selected in step (g) and with a suboptimal dose of a CREB function stimulating agent;

(i) assessing endogenous CREB-dependent gene expression in the cells which have been contacted with the candidate compound and with the CREB function stimulating agent;

(j) comparing endogenous CREB-dependent gene expression assessed in step (i) with endogenous CREB-dependent gene expression in control cells which have been contacted with said CREB function stimulating agent and which have not been contacted with the candidate compound;

(k) selecting said candidate compound if: (1) endogenous CREB-dependent gene expression assessed in step (i) is increased relative to endogenous CREB-dependent gene expression in control cells which have been contacted with said CREB function stimulating agent and which have not been contacted with the candidate compound; and (2) endogenous CREB-dependent gene expression in control cells which have not been contacted with the CREB function stimulating agent and which have been contacted with the candidate compound is not significantly different relative to the CREB-dependent gene expression in control cells which have not been contacted with said CREB function stimulating agent and which have not been contacted with said candidate compound, thereby selecting a confirmed candidate compound;

(l) administering the confirmed candidate compound selected in step (k) to an animal;

(m) training the animal administered the confirmed candidate compound under conditions appropriate to produce long term memory formation in the animal;

(n) assessing long term memory formation in the animal trained in step (m); and

(o) comparing long term memory formation assessed in step (n) with long term memory formation produced in the control animal to which the confirmed candidate compound has not been administered.

The methods disclosed by Son *et al.* in Figures 1, 2 and 3 do not include any of steps (a) to (o) recited in independent Claim 19. The method disclosed by Son *et al.* in Figure 4 does not include any of steps (b) to (o), and in particular, does not include steps (e) to (o), recited in Claim 19. Importantly, Son *et al.* do not teach or suggest a method comprising steps (a) to (o) recited in Claim 19. As such, Claims 19-25 must be considered novel over the Son *et al.* reference.

Inventive Step

Claims 1-25 have been rejected under PCT Article 33(3) as being obvious over Sheriff *et al.*, Ying *et al.*, Kang *et al.* and Son *et al.*

The cited references are discussed above. None of the cited references teach or suggest methods for identifying or screening for cognitive enhancers comprising a primary screen, a secondary screen, and a tertiary screen, where the primary screen is a cell-based method used to identify candidate compounds, the secondary screen is a cell-based method used to identify confirmed candidate compounds, and the tertiary screen is a behavioral model used to identify cognitive enhancers. Importantly, none of the cited references provide motivation to develop methods for identifying or screening for cognitive enhancers comprising a primary screen, a secondary screen, and a tertiary screen. Additionally, none of the cited references teach or suggest contacting cells comprising a indicator gene operably linked to a CRE promoter or cells of neural origin with a test compound and a *suboptimal dose* of a CREB function stimulating agent.

Therefore, it would not have been obvious to one of ordinary skill in the art to develop a method for screening a compound for its ability to enhance CREB pathway function comprising steps (a) to (o) as recited in Claim 19 with a reasonable expectation of success. It would not have been obvious to one of ordinary skill in the art to develop a method for identifying candidate compounds for enhancing CREB pathway function comprising steps (a) to (f) as recited in Claim 1 with a reasonable expectation of success. It would not have been obvious to one of ordinary skill in the art to develop a method for assessing the effect on CREB-dependent gene expression of a candidate compound for

enhancing CREB pathway function comprising steps (a) to (c) as recited in Claim 12 with a reasonable expectation of success.

Accordingly, Claims 1-25 must be considered as involving an inventive step over the cited references.

CONCLUSION

In view of the above amendments and remarks, favorable consideration of the application is respectfully requested.

Respectfully submitted,

HAMILTON, BROOK, SMITH & REYNOLDS, P.C.

By _____

Helen Lee
Registration No. 39,270
Telephone (978) 341-0036
Facsimile (978) 341-0136

Concord, Massachusetts 01742-9133
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12. A method for assessing the effect on CREB-dependent gene expression of a candidate compound for enhancing CREB pathway function comprising the steps of:

a) contacting cells of neural origin with a candidate compound and with a suboptimal dose of a CREB function stimulating agent;

b) assessing endogenous CREB-dependent gene expression in the cells which have been contacted with said candidate compound and with said CREB function stimulating agent; and

c) comparing endogenous CREB-dependent gene expression assessed in step b) with endogenous CREB-dependent gene expression in control cells which have been contacted with said CREB function stimulating agent and which have not been contacted with said candidate compound.

13. The method of Claim 12 wherein said cells of neural origin are contacted with said candidate compound prior to contact with said CREB function stimulating agent.

14. The method of Claim 12 wherein said cells of neural origin are neurons.

15. The method of Claim 14 wherein said neurons are primary hippocampal cells.

16. The method of Claim 12 wherein said CREB function stimulating agent is forskolin.

17. A method for assessing the effect on long term memory formation in an animal of a candidate compound for enhancing CREB pathway function comprising the steps of:

a) administering said candidate compound to be assessed to said animal, said candidate compound identified according to the method of Claim 7;